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FUSION PROTEINS

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(57) Claim

1. Particles composed of a fusion protein comprising HBCAg to the amino terminus of which is linked a heterologous antigenic epitope, excluding the first eight amino-acids of β -Galactosidase, which epitope is exposed on the outer surface of the particles.

9. A DNA sequence encoding a fusion protein comprising HBCAg to the amino terminus of which is linked a heterologous antigenic epitope, excluding the first eight amino-acids of β -Galactosidase.

5. Particles according to claim 4, wherein the antigenic epitope is an epitope of foot-and-mouth disease virus, poliovirus, human rhinovirus, influenza virus or hepatitis B virus surface antigen.

7. A vaccine comprising, as active ingredient, particles as claimed in any one of the preceding claims and a physiologically acceptable carrier or diluent.

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Section 29
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Section 29(1)
Regulation 3.1(2)

AUSTRALIA

Patents Act 1990

PATENT REQUEST AND NOTICE OF ENTITLEMENT

We THE WELLCOME FOUNDATION LIMITED
of Unicorn House, 160 Euston Road, London NW1 2BP, ENGLAND
being the Applicant and Nominated Person, request the grant of a patent for an invention entitled "Fusion Proteins" which is described in the complete specification of Application No. 49273/90.

Application No. 49273/90 is a further application made of Application No. 69792/87 in the name of The Wellcome Foundation Limited under the provisions of Section 51(1) of the Patents Act 1952 in respect of an invention disclosed in the complete specification.

The name and address of each actual inventor of the invention is as follows:

Peter Edmund HIGHFIELD, Berwyn Ewart CLARK and Anthony Robin CARROLL: of Langley Court, Beckenham, Kent BR3 3BS, England; Ash Road, Pirbright, Woking, Surrey, England and Ash Road, Pirbright, Woking, Surrey, England respectively.

The inventors are the applicants of the basic application No. 012948 filed in the United States of America on 10 February 1987.

The nominated person is the assignee of the invention from the inventor.

For the purposes of Section 40, the specification relies on Section 6 of the Act.

Depositor	Deposit No	Lodgement Date	Depository Institution
The Welicome Foundation Limited	NCIB12423	6 March 1987	National Collection of Industrial Bacteria

The nominated person has entitlement from the depositor to rely upon the deposit listed above.

The basic application is the application first made in a Convention country in respect of the invention.

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DATED this 13th day of August 1993

By their Patent Attorney

Chris Clegg

642859

COMMONWEALTH OF AUSTRALIA

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COMPLETE SPECIFICATION

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Complete Specification for the invention entitled:

FUSION PROTEINS

The following statement is a full description of this
invention, including the best method of performing it known to
us:-

15431-L:ADK

3687A:rk

TITLE: FUSION PROTEINS

This invention relates to the construction of fusion proteins.

5 Hepatitis B virus is a DNA virus with a partly double stranded genome of 3200 nucleotides. The viral DNA is surrounded by the viral coded core antigen (HBcAg) which is enclosed by the similarly coded surface antigen (Robinson, 1977). Removal of the surface antigen by mild detergent treatment leaves a core particle 27nm in diameter composed of HBcAg and the viral DNA. HBcAg has been 10 expressed in microbial cells as the native polypeptide and as a derivative fused to the terminal eight residues of beta-galactosidase (see Murray et al, 1984 for refs).

When synthesized in E. coli the core protein self assembles into 27 nm particles which can be visualized 15 under the electron microscope (Cohen and Richmond, 1982) and which are immunogenic in laboratory animals (Stahl et al, 1982). The amino acid sequence of the core antigen shows a region towards the carboxy terminus which is homologous with that found in protamines (DNA binding proteins). By

20 inference, it has been suggested that this part of the molecule interacts with DNA during assembly of core particles (Pasok et al, 1979).

We have previously shown that it is possible to express immunogenic epitopes of foot-and-mouth disease

virus (FMDV) as fusion proteins to beta-galactosidase in bacterial systems and also in cells infected with recombinant vaccinia virus (Winther et al, 1986; Newton et al, 1986). Since beta-galactosidase in its active form exists as a tetrameric structure, the nature of the fusion was such that only four copies of the FMDV sequence would be present on each complex but these only represent about 2% of the weight of the fusion protein. Further, animals vaccinated with the recombinant vaccinia virus failed to produce neutralizing antibody (Newton et al, 1986). The reason for this poor response may be because beta-galactosidase is expressed in the cytoplasm.

In order to improve the presentation of FMDV epitope to the immune system, we have fused the FMDV sequence to the HBcAg. DNA sequences were constructed, each encoding a fusion protein comprising HBcAg to the amino terminus of which was linked FMDV VP1 residues 141 to 160. The fusion gene sequence was incorporated into the vaccinia virus genome. Cells infected with the recombinant virus expressed the fusion protein strongly. The recombinant protein self-assembled into particulate structures. These have a high density of externally located FMDV epitopes. By expressing the FMDV epitope in this way, it is possible to present it in a more virus-like form.

This approach for presenting antigenic epitopes

to the immune system has general applicability. Accordingly, the present invention provides a DNA sequence encoding a fusion protein comprising EBCAg to the amino terminus of which is linked a heterologous antigenic epitope.

For expression of the fusion protein, the DNA sequence is incorporated in an expression vector. The DNA sequence is incorporated in a vector such that the vector, when provided in a suitable prokaryotic or eucaryotic host, is capable of expressing the fusion protein. The vector may be a plasmid. Alternatively, the vector may be a viral vector which incorporates the DNA sequence such that the fusion protein is expressed by cells infected with the vector. The fusion protein is obtained in particulate form. The fusion protein is used as a vaccine. Vaccines may therefore comprise the fusion protein and a physiologically acceptable carrier or diluent. In view of the apparent involvement of the carboxy terminus of the EBCAg in DNA interaction at the centre of the hepatitis B virus, the heterologous epitope is fused to the amino terminus of the EBCAg. The epitope may be fused directly to the EBCAg. Alternatively, the heterologous epitope may be fused to the EBCAg via an intervening linker. Such a linker may be composed of one or more, for example up to ten, amino acid residues. The precore EBCAg signal amino acid sequence, which normally is located immediately before the amino terminus of the EBCAg,

may therefore be absent from the fusion protein or part of it may comprise the linker. To the amino-terminus of the heterologous epitope may be located one or more amino acid residues prior to a Met residue corresponding to a translational start codon.

Any heterologous antigenic epitope may be fused to the EBcAg. By "heterologous" is meant an epitope which is not an epitope of EBcAg. The size of the epitope may be from four to twenty six amino acid residues. Two or more heterologous antigenic epitopes may be provided. In this way, polyvalent vaccines can be presented.

The heterologous epitope may be that of a virus, bacterium or protozoan. As examples of viral epitopes, there may be mentioned those of FMDV, poliovirus, human rhinovirus, influenza virus and hepatitis B virus surface antigen (HBsAg). Protozoan whose epitopes may be provided include the malaria parasite Plasmodium falciparum.

The major FMDV antigenic sites correspond to amino acid residues 141 to 160 and 200 to 213 of the VP1 capsid protein. Either or both of these sequences of amino acid residues may therefore constitute the heterologous antigenic epitope(s). Alternatively, parts of these sequences may be provided e.g. residues 142 to 145, 146 to 151, 142 to 151 or 142 to 160. A suitable DNA construct incorporating VP1 amino acid residues 142 to 160 of FMDV type O, Kaufbeuren, and its corresponding amino acid sequence as denoted by the one-letter code, is shown below.

T T T T T T C T A T G C T A T A A A T G A A T T C A G, C T
P11k Promoter M N S A

C C G A A C C T G C G T G G T G A C C T G C A G G T T C T G
S P N L R G D L Q V L
(VP1

G C T C A G A A A G T T G C T C G T A C C C C T G C C C G G G A
A Q K V A R T L P G
10 VP1] [
G C T C C G G A T C C G G C G C C C T T G G G T G G C T T
A P D P R A L G W L
LINKER] [

15

T G G G G C A T G G A C A T T G A C C C T T A T A A A G A A
W G M D I D P Y K E
HEPATITIS B CORE ANTIGEN

T T T -----

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F

The DNA sequence encoding the fusion protein can
be prepared starting from a DNA sequence encoding HBcAg,
for example a plasmid incorporating the HBcAg gene. A DNA
sequence encoding the heterologous antigenic peptide to be
25 incorporated into the fusion protein can be

included in the fusion protein is ligated in the correct frame to the 5' end of the HBcAg gene. A vector capable of expressing the fusion protein may be prepared by incorporating a DNA sequence encoding the fusion protein 5 between translational start and stop signals in a vector and providing a promoter for the sequence. By transforming suitable host cells with such an expression vector, the fusion protein can be produced.

A viral vector capable of expressing the fusion 10 protein may therefore be prepared by incorporating a DNA sequence encoding the fusion protein between translational start and stop signals in the genome of a virus and providing a promoter for the sequence. Typically, this may be achieved by:

- 15 (i) constructing a shuttle vector which incorporates, under the transcriptional control of a promoter, a DNA sequence encoding the fusion protein between translational start and stop signals; and
- (ii) transfecting with the shuttle vector and 20 infecting with a virus mammalian cells such that the DNA sequence and the promoter are incorporated in the viral genome.

The DNA sequence comprises DNA encoding the heterologous antigenic epitope immediately upstream of and 25 in the correct frame in relation to the HBcAg gene. The DNA sequence and promoter are incorporated in the viral genome by homologous recombination. Appropriate flanking

sequences of viral DNA are therefore provided on either side of the DNA sequence and promoter in the shuttle vector. The fusion protein is expressed by cells infected with the resultant recombinant virus.

5 The shuttle vector is typically a plasmid. It comprises a bacterial origin of replication to enable the manipulation required in step (i) to be carried out in bacteria, especially E. coli. The promoter is typically a viral promoter, more preferably a promoter endogenous to
10 the virus into the genome of which the DNA encoding the fusion protein is to be inserted. The heterologous antigenic epitope is generally prepared by chemical synthesis and/or by cloning. A linker DNA sequence may be provided between the heterologous epitope and the HBcAg.

15 A vaccinia virus system may be used. A shuttle vector may be constructed in which the fusion protein gene is incorporated under the transcriptional control of a vaccinia promoter. A suitable promoter is the vaccinia pllk promoter. The promoter and fusion protein gene are flanked by vaccinia virus DNA which is not essential for
20 virus replication. Typically, flanking segments of the vaccinia gene for thymidine kinase (TK) are used. The fusion protein gene is present between DNA encoding translational start and stop signals.

25 The vaccinia promoter and fusion protein gene are then inserted into the vaccinia genome by homologous

recombination. This is typically achieved by infecting mammalian cells with vaccinia virus such as the Wyeth (US vaccine) strain and also transfecting the cells with the shuttle vector. The site of insertion is determined by the flanking vaccinia DNA segments of the shuttle vector. By means of homologous recombination the functional TK gene of the wild-type virus is replaced by the non-functional TK gene sequence included within which is the fusion protein gene. The resulting recombinant virus is TK⁻ and can therefore be selected accordingly.

The fusion protein incorporating the antigenic epitope is expressed in cells, typically mammalian cells as below, infected with the recombinant viral vector. The fusion protein is obtained from the cells in conventional manner e.g. by lysing the cells followed by centrifugation. The fusion protein self-assembles within the cells to form particles. These particles closely resemble the 27 nm core particles composed of EDCAg and viral DNA which can be obtained by denaturing hepatitis B virus. The heterologous epitopes are exposed on the outer particle surface.

The fusion protein may also be obtained using other expression systems. Expression may be achieved in any other suitable host, prokaryotic or eucaryotic. This means that a compatible host must be selected, for example one in which the fusion protein is neither degraded by endogenous proteases nor toxic to the host. Such a host may be selected by a person skilled in the art, with

routine experimentation if necessary. We in fact were unable to produce a FMDV-BBcAg fusion protein in E. coli. The problem is apparently one of toxicity to the bacterium of the FMDV sequences which we used. BBcAg by itself can 5 be expressed stably in E. coli. It is therefore the effect of the extra sequence comprising the heterologous antigenic epitope on the E. coli which appears to be controlling.

As a prokaryotic host, E. coli strains may be employed. Other microbial strains which may be used are 10 bacilli such as Bacillus subtilis and other enterobacteriaceae such as Salmonella typhimurium or Serriatia marcescens. Various pseudomonas species may be used. Plasmid vectors are typically used to transform such hosts. The plasmids generally contain an origin of 15 replication and control sequences which are derived from species compatible with the host. Marking sequences capable of providing phenotypic selection in transformed cells are ordinarily present in the plasmid.

Cells from vertebrates or invertebrates, 20 preferably mammalian cells, can be used as eucaryotic host cell lines. Cell lines such as VERO, HeLa, CHO (Chinese hamster ovary), WI38, BHK, COS-7, MDCK and CV-1 may be employed. Expression vectors for such cells generally contain an origin of replication, a promoter located in 25 front of the gene to be expressed, and any necessary

ribosome binding sites, RNA splice sites, polyadenylation site and transcriptional terminator sequences. Viral promoters preferably are employed. Viral vectors as above may be used, such as a baculovirus expression system.

5 Eucaryotic microbes such as yeast cultures may alternatively be used as host cells. Saccharomyces cerevisiae strains can therefore be employed. A plasmid vector such as plasmid YRp7 is typically utilised to transform such hosts. Any plasmid vector containing a 10 yeast-compatible promoter, origin of replication and termination sequences is suitable.

The fusion protein may be used as a vaccine for a human or animal. It may be administered in any appropriate fashion. The choice of whether an oral route or a 15 parenteral route such as sub-cutaneous, intravenous or intramuscular administration is adopted and of the dose depends upon the purpose of the vaccination and whether it is a human or mammal being vaccinated. Similar criteria govern the physiologically acceptable carrier or diluent 20 employed in the vaccine preparation. Conventional formulations, carriers or diluents may be used. Typically, however, the fusion protein is administered in an amount of 10-1000 ug per dose, more preferably from 10-100 ug per dose, by either the oral or the parenteral route.

25 The following Example illustrates the present invention. In the accompanying drawings:

Figure 1 shows the construction of plasmid pFOHc; Figure 2 shows the shuttle vector p_vFOHC, the sequence of which contains two in-phase initiation codons separated by the FMDV VP1₁₄₂₋₁₆₀ sequence and six amino acids of the authentic HB "pre-core" sequence; Figure 3 presents the results of sandwich ELISA of cell lysates from wild-type (Wyeth) or recombinant (vFOHC) infected cells; Figure 4 shows the sucrose gradient profile of core reactive material; and Figure 5 shows the results of labelling wild-type-vaccinia virus and recombinant (vFOHC) infected cells with ³⁵S-methionine and analysing the cellular supernatants by immunoprecipitation with HBCAg reactive antisera and polyacrylamide gel electrophoresis (PAGE).

EXAMPLE

Two clones were used to construct the fusion protein described in this study. One clone representing hepatitis B core antigen (HBCAg) was obtained from Dr. P. Highfield (pWRL 3123). This clone had been modified at the NH₂ terminus such that it could be expressed in bacteria as a fusion protein to the E. coli protein TRP E. E. coli HB101 harbouring pWRL 3123 was deposited at the National Collection of Industrial Bacteria, Aberdeen, GB on 6 March 1987 under accession number NCIMB 12423. A second clone representing FMDV 142-160 sequences from O, Kaufbeuren linked to the amino terminus of β -galactosidase was obtained from Dr. M. Winther (pWRL 201) (Winther et al, 1986). Restriction maps of each clone are shown in Figure

1. As can be seen in Figure 1, the junction between the FMDV sequence and the β -galactosidase comprises a Bam HI restriction site. The strategy undertaken therefore involved the fusion of the FMDV sequence and the HBcAg sequence through this Dcm EI site.

The initial stage in the construction therefore involved insertion of a synthetic oligonucleotide linker for Bam HI at the 5' end of the HBcAg gene of pWRL 3123.

10 The site used for insertion of the linker was the Nar I site at position 290. However a second Nar I site at position 1230 was also present in this plasmid. The plasmid was therefore partially digested with Nar I so that a population of plasmid molecules which had been cut at 15 only one Nar I site could be observed by agarose gel electrophoresis and purified. After flush ending the Nar I sites using the Klenow fragment of DNA polymerase I, a synthetic oligonucleotide linker representing a Bam HI site was ligated into the partial Nar I digest and the resulting 20 plasmids were used to transform E. coli. Clones were then analysed for the presence of a Dcm EI linker in the correct Nar I site by restriction mapping.

One such clone, designated pEB208, was isolated and DNA prepared. The length of the Dcm EI linker had been 25 specifically chosen so that, when ligated to the FMDV portion of pWRL201 (Fig 1), the translational reading frame

would be continuous and a fusion protein could be produced. Concomitant with the insertion of the Bam HI linker, the Nar I site into which it had been inserted, was destroyed. It was therefore possible to remove the HBcAg sequence from 5 pEB208 by Dan EI - Nar I digestion whereupon a DNA fragment of 960 bases was produced. Similarly a Bam HI - Nar I fragment from pHRL201 of approximately 3.5 kilobases was purified. These two fragments were ligated together and the correct clone (pFOHc) was identified by restriction 10 mapping.

As can be seen from Figure 1, pFOHc can be expressed in bacterial cells under the control of the tac promoter. In order to facilitate the transfer of the hybrid gene to a vaccinia virus (VV) shuttle vector, 15 however, plasmid pFOHc was cut at the single Nar I site and a second EcoRI site was introduced as a synthetic linker. This enabled the complete hybrid gene to be isolated as an EcoRI fragment.

The VV shuttle vector was pVp1lk which was 20 derived from the vector pE3JΔR1A (Newton et al, 1986) by deletion of extraneous VV sequences. This shuttle vector has a VV promoter (in this case p1lk) inserted into VV thymidine kinase (TK) gene. This vector has a unique EcoRI site immediately following the VV p1lk promoter and AUG 25 (Bertholet et al, 1985). The Ec RI site and AUG are in the same translational reading frame as the amino terminal

EcoRI site of the hybrid gene in pFOHC. The FMDV-HBcAg gene was therefore inserted as the EcoRI fragment into EcoRI cut dephosphorylated pVplik. Clones with the hybrid gene in the correct orientation relative to the plik promoter were identified by restriction mapping. This clone was designated pVFOHC (Figure 2).

This shuttle plasmid was then inserted into the genome of the Wyeth (US vaccine) strain of VV, under the control of the plik promoter, by homologous recombination using the flanking TK sequences (Mackett et al, 1985 a and b). Individual progeny plaques with a TK⁻ phenotype were screened for the presence of FMD-HBcAg DNA by dot blot hybridisation.

CV-1 Cell lysates from wild-type (Wyeth) and recombinant (vFOHC) infected cells were screened for the presence of core antigen and for FMDV sequences by sandwich ELISA. Antigen from infected cells was bound to ELISA plates using either FMD virus particle (146S) or FMD VP1 141-160 antisera raised in rabbits. Each trapped antigen was then assessed for the presence of either HBc, FMD 146S or FMD VP1 142-160 epitopes by binding with the respective guinea pig antisera and development with anti guinea pig peroxidase conjugate. The results are shown in Figure 3. As can be seen in Fig 3, a protein recombinant from (vFOHC) infected cell lysates was trapped with anti-FMDV 141-160 antiserum and this protein could then react with anti HBc,

anti-FMDV 141-160 and FMDV antivirion serum in a sandwich ELISA.

Furthermore, this protein could be purified by ultracentrifugation suggesting that it was particulate in nature. This was illustrated more clearly when the products of centrifugation were sedimented on a sucrose density gradient and fractions were re-assayed for the presence of core antigen by ELISA. Cell lysates from recombinant (vFOKc) vaccinia virus infected cells or bacteria expressing native core antigen were fractionated on 15-45% sucrose gradients. Fractions were assayed for the presence of core reactive material by indirect sandwich ELISA using human anticore antiserum as trapping antibody and guinea pig EEc antigen antiserum for detection. The results are shown in Figure 4. The position at which FMD virus sediments is also indicated. Fig 4 shows that a peak of EEcAg reactive material was observed in a position similar to that observed when core particles expressed in bacteria were centrifuged in parallel. Thus it appears that the presence of the FMDV VP_{1,41-160} sequence does not interfere with the particulate nature of the core particles.

The ability of the fusion protein to self assemble into regular, 27nm core like particles was confirmed by electron microscopic examination of immune complex s formed with sucrose gradient purified material.

The complexes were formed by reacting the FMDV-HBcAg particles with antiserum raised to intact foot and mouth disease virus. The complexes were adsorbed to form over coated grids and negatively stained with phosphotungstic acid. As was to be expected from the ELISA data shown in Fig 3, immune complexes were also seen after reacting the particles with antisera to HBcAg or to synthetic FMDV peptide 141-160.

Finally, the nature of the polypeptides synthesized in CV-1 cells infected with wild-type vaccinia virus or vFOPC by labelling the cells with 35 S-methionine and analysing the cellular supernatants by immunoprecipitation with HBcAg reactive antisera and PAGE. Infected cells were pulse labelled with 35 S-methionine for 1 hour and total cell lysates prepared. Immunoprecipitation of labelled proteins was carried out with vaccinia virus antiserum (A,D), human HepB antiserum (B,E) or guinea pig EBC antigen antiserum (C,F). Precipitated proteins were then analysed by PAGE and fluorography. The results are shown in Figure 5. The position of the FMDV VP_{1₁₄₁₋₁₆₀} -HBcAg fusion protein is arrowed. As shown in Fig 5, several proteins are specifically precipitated from extracts of recombinant (vFOPC) infected cells by EBCAg antiserum. Two of these proteins (Mol. Wt. 25KD and 20 KD) represent the complete fusion protein and a derivative having lost a 5KD fragment.

from its carboxy terminus by proteolytic digestion which would correspond to FMDV VP_{1,43-160}-HBeAg (Mackay et al, 1981).

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Particles composed of a fusion protein comprising HBcAg to the amino terminus of which is linked a heterologous antigenic epitope, excluding the first 5 eight amino-acids of β -Galactosidase, which epitope is exposed on the outer surface of the particles.
2. Particles according to claim 1, wherein the heterologous antigenic epitope is fused directly to the HBcAg.
- 10 3. Particles according to claim 1, wherein the heterologous antigenic epitope is fused to the HBcAg via a linker of from one to ten amino acid residues.
- 15 4. Particles according to any one of the preceding claims, wherein the heterologous antigenic epitope is an epitope of a virus, bacterium or protozoan.
- 20 5. Particles according to claim 4, wherein the antigenic epitope is an epitope of foot-and-mouth disease virus, poliovirus, human rhinovirus, influenza virus or hepatitis B virus surface antigen.
- 25 6. Particles according to claim 4, wherein the antigenic epitope is an epitope of Plasmodium falciparum.
7. A vaccine comprising, as active ingredient, particles as claimed in any one of the preceding claims and a physiologically acceptable carrier or diluent.
- 25 8. A method of vaccinating a human or animal, which method comprising the step of administering thereto an effective amount of particles as claimed in any one of claims 1 to 6.



9. A DNA sequence encoding a fusion protein comprising HBcAg to the amino terminus of which is linked a heterologous antigenic epitope, excluding the first eight amino-acids of β -Galactosidase.

5 10. A DNA sequence according to claim 9, wherein the heterologous antigenic epitope is fused directly to the HBcAg.

10 11. A DNA sequence according to claim 9, wherein the heterologous antigenic epitope is fused to the HBcAg via a linker of from one to ten amino acid residues.

12. A DNA sequence according to any one of claims 9 to 11, wherein the heterologous antigenic epitope is an epitope of a virus, bacterium or protozoan.

15 13. A DNA sequence according to claim 12, wherein the antigenic epitope is an epitope of the foot-and-mouth disease virus, poliovirus, human rhinovirus, influenza virus or hepatitis B virus surface antigen.

20 14. A DNA sequence according to claim 12, wherein the antigenic epitope is an epitope of Plasmodium falciparum.

15. A vector which incorporates a DNA sequence as claimed in any one of claim 9 to 14 and which is capable, when provided in a suitable host, of expressing the said fusion protein.

25 16. A vector according to claim 15, which is a



viral vector.

17. A vector according to claim 16, which is a recombinant vaccinia virus which incorporates the said DNA sequence.

18. A vector according to claim 15, which is a 5 plasmid.

19. A host in which is provided a vector as claimed in any one of claims 15 to 18.

20. A host according to claim 19 in which the vector is a viral vector and the host is a mammalian cell 10 line.

21. A process for the preparation of a vector as defined in claim 15, which process comprises incorporating a DNA sequence as claimed in any one of claims 9 to 14 between translational start and stop signals in a vector 15 and providing a promoter for the sequence.

22. A process according to claim 21, wherein the vector is a virus.

23. A process according to claim 22, comprising:

(i) constructing a shuttle vector which

20 incorporates, under the transcriptional control of a promoter, a DNA sequence encoding the fusion protein between translational start and stop signals; and

(ii) transfecting with the shuttle vector and infecting with a virus mammalian cells such that the DNA 25 sequence and the promoter are incorporated in the viral genome.

24. A process according to claim 23, wherein the virus is a vaccinia virus.

5 25. A process for the preparation of particles as claimed in any one of claims 1 to 6, which process comprises culturing a host as claimed in claim 19 or 20 such as to cause expression of the fusion protein and thereby formation of said particles.

10 26. A process for the preparation of a vector as defined in claim 15, said process being substantially as hereinbefore described in the Example.

27. A process for the preparation of particles as claimed in any one of claims 1 to 6, said process being substantially as herein before described in the Example.

Dated this 2nd day of September 1993

15

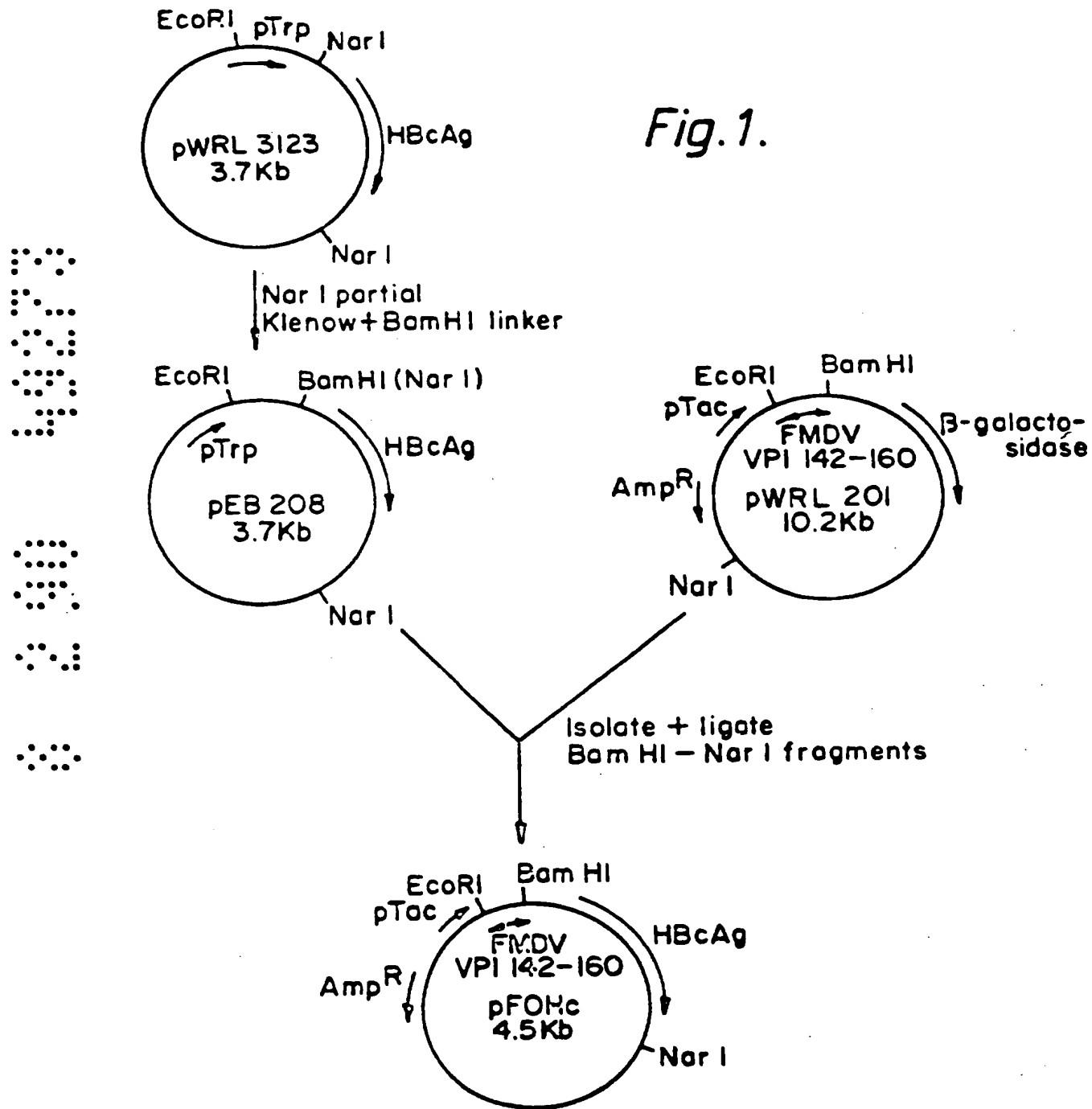
THE WELLCOME FOUNDATION LIMITED

By their Patent Attorneys

GRIFFITH HACK & CO



Fig. 1.



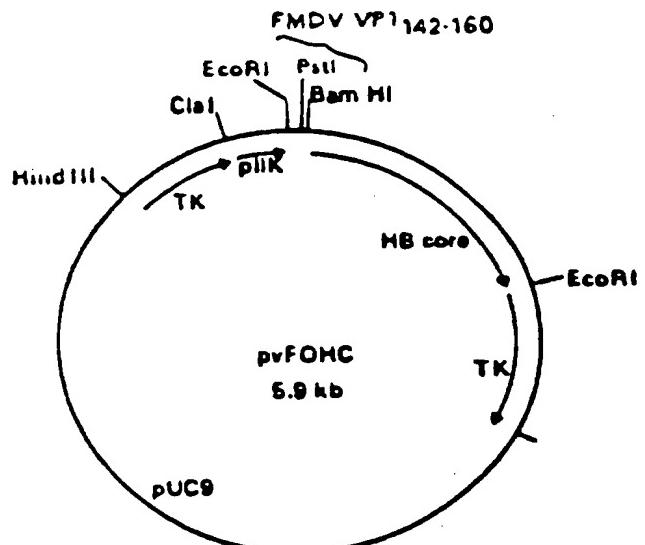


Fig.2.

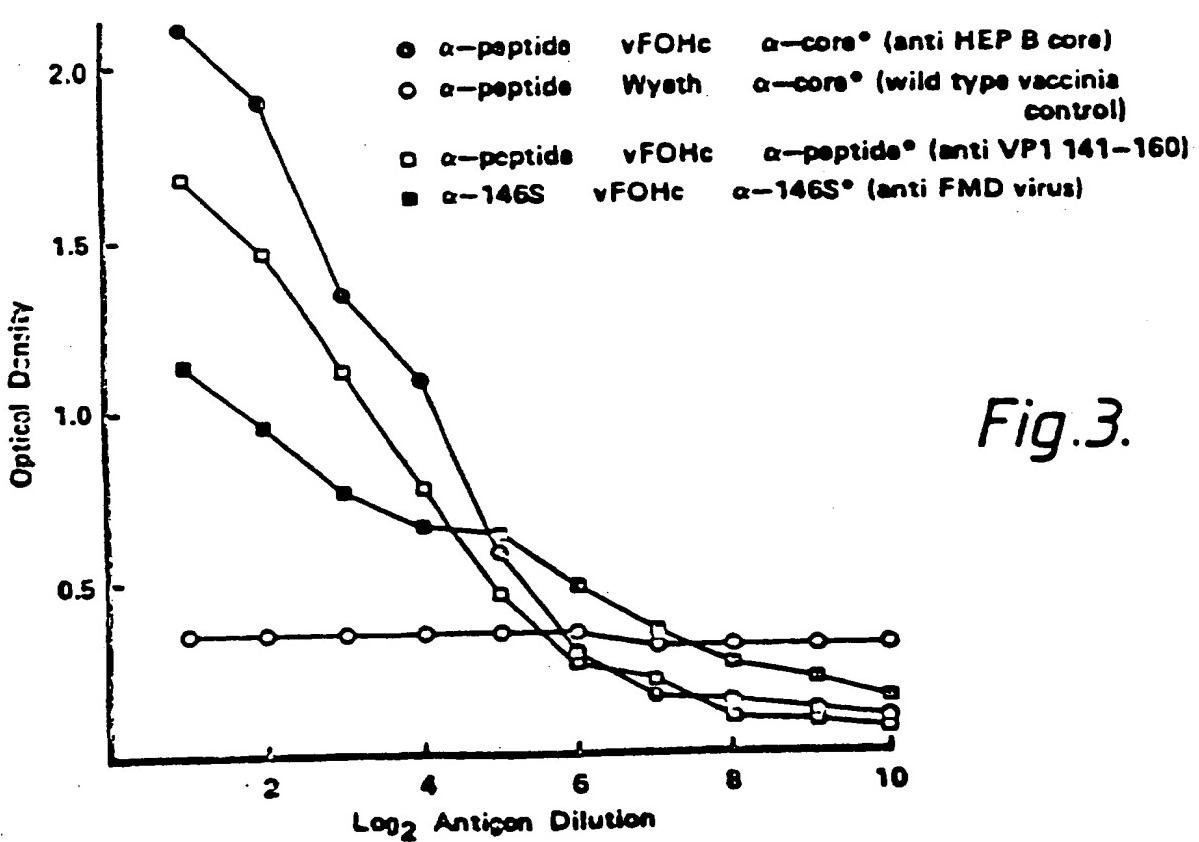
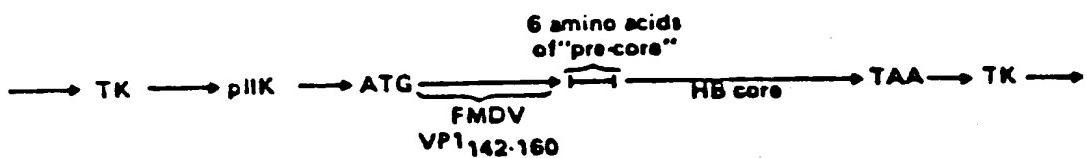
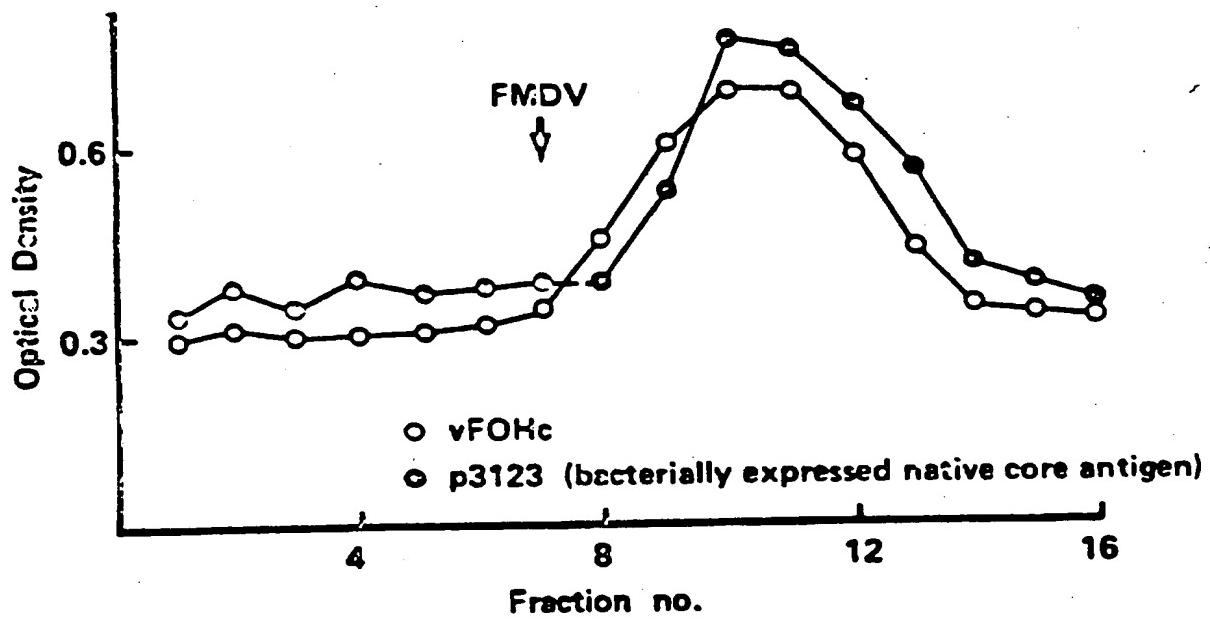


Fig.3.

Fig. 4.



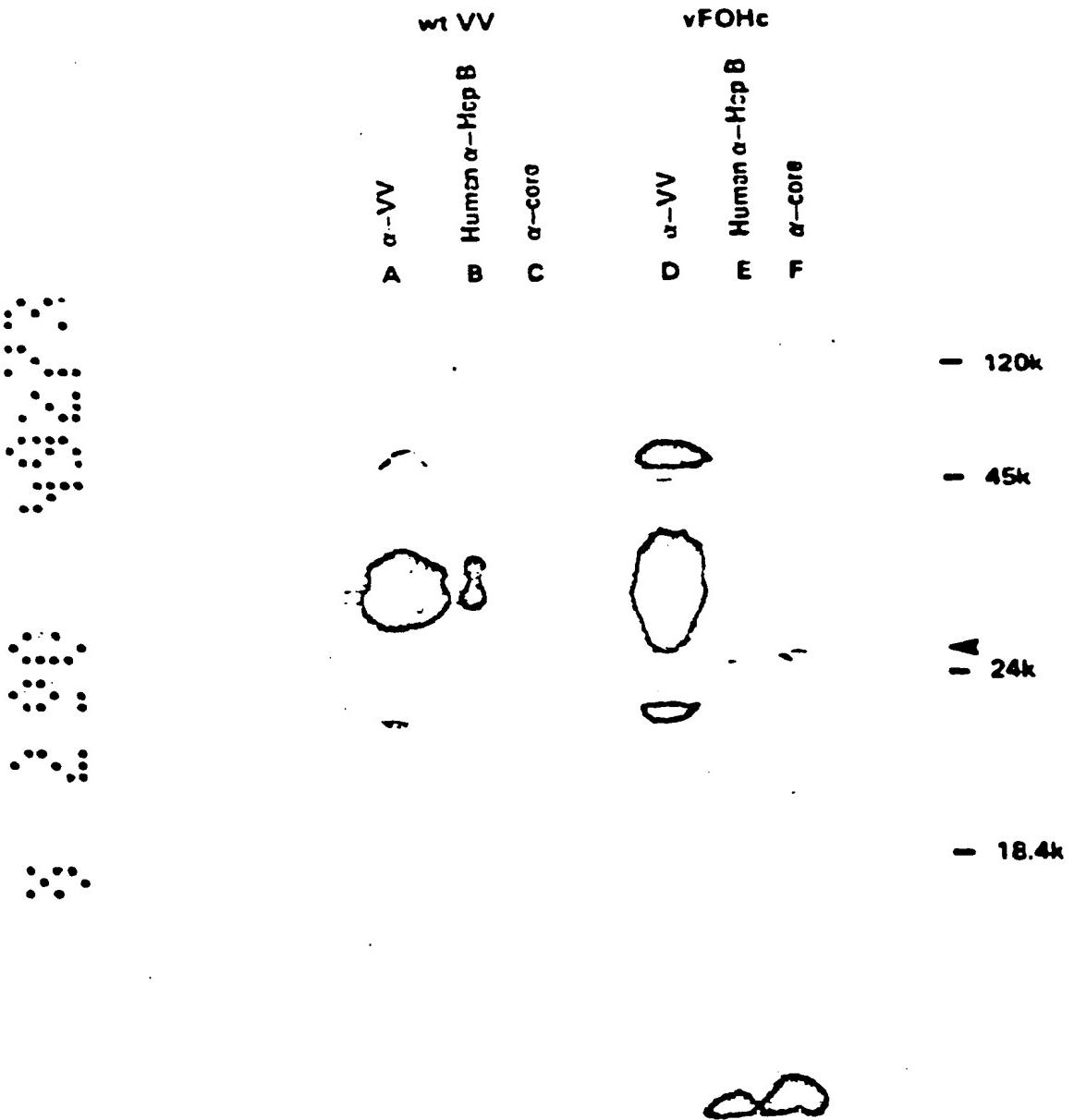


Fig. 5.